THE MYOSIN OF DEVELOPING AND DYSTROPHIC SKELETAL MUSCLE

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1. Introduction

Perry [1] has proposed that a reversal of the normal developmental process occurs during some types of muscular dystrophy in that certain isoenzymes are found in the proportions typical of foetal tissue. In the case of dystrophy induced by vitamin E deficiency in rabbits [2], the specific Ca^{2+}-ATPase activity of adult myosin decreased to a lower level characteristic of foetal myosin preparations and this was correlated with a reduction in the relative amount of the fastest migrating of the light chains of myosin detected by gel-electrophoresis in 8 M urea. However, Dow and Stracher [3] found no significant differences between the specific Ca^{2+}-ATPase activity of adult and foetal myosin of chicken. SDS-gel electrophoresis showed that there were three light chains in adult chicken myosin and only the two largest of these three in foetal myosin but it was demonstrated by experiments in which heavy and light polypeptide chains were dissociated and recombined or cross hybridized, that only two light chains are required for the expression of the full Ca^{2+}-ATPase activity of myosin [3]. The smallest chain in SDS-gel electrophoresis (L_3) corresponds to the fastest migrating chain in 8 M urea-gel electrophoresis [4].

In the present investigation, the specific Ca^{2+}-ATPase activity of myosin prepared from the mixed skeletal muscle of the hind limbs and pelvic region of adult, foetal and dystrophic mice was related to the subunit composition analyzed by SDS-gel electrophoresis. The results indicate that different levels of Ca^{2+}-ATPase activity can be correlated with different proportions of the three light chain components (L_1, L_2 and L_3) of myosin. Specific Ca^{2+}-ATPase levels of foetal and dystrophic myosin were lower than those of adult myosin prepared in the same experiment. Foetal myosin contained only 0.6 moles of the light chain L_3 suggesting that the preparation may contain some myosin molecules without any L_3 chains at all. Dystrophic myosin contained a similarly reduced amount of L_3 but the proportion of total light chains was also decreased.

2. Materials and methods

Myosin was prepared from muscle dissected from the diseased hind limbs and pelvic region of 6–10 week-old adult dystrophic mice (strain 129 RE – ref. [5] and also from the same area in normal litter mates and adult Banded-Dutch mice. (There was no detectable difference between myosin from normal 129RE adult mice and Banded-Dutch mice). Foetuses (19-day in utero) were taken from Banded-Dutch mothers and the whole eviscerated hind regions of the carcasses used to prepare foetal myosin.

The method for the preparation of myosin was based on several procedures [3, 6, 7] and included the following steps: (1) initial extraction with 0.01 M sodium pyrophosphate; 0.005 M $MgCl_2$; 0.5 M KCl; 0.05 M phosphate pH 6.5 to reduce actomyosin extraction. (2) Treatment with ribonuclease A to reduce ribonucleoprotein contamination. (3) Ammonium sulphate fractionation to obtain the myosin contained in the 38–50% saturation fraction. (4) Ion-exchange

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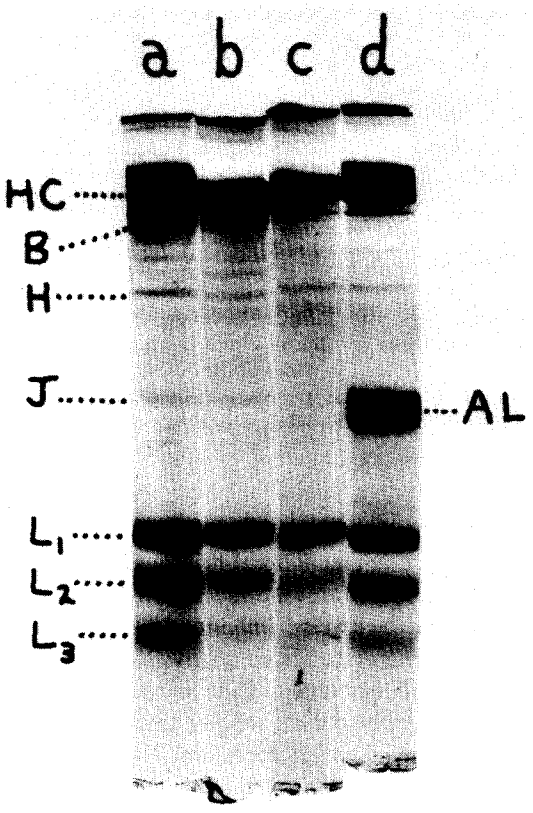

Fig. 1. Electrophoresis of myosins on SDS-polyacrylamide gels. 1 mg quantities of protein (including MW markers) were dissolved in 1 ml of 8 M urea, 1% SDS, 1% β-mercaptoethanol, 0.01 M sodium phosphate buffer pH 7.0 and incubated at 37°C for 2 hr. 10–20 μg were electrophoresed for 4 hr at 8 mA per gel through gels containing 10% acrylamide and 0.134% methylene bisacrylamide (half the concentration of bisacrylamide recommended by Weber and Osborn [8]) in 0.075 M sodium phosphate buffer pH 7.0, 0.1% SDS. Gels were stained in 0.2% Coomassie Blue in 10% acetic acid, 45% ethanol at 60°C for 2 hr, and destained in several changes of 10% acetic acid, 25% ethanol, and 10% acetic acid at 60°C. Molecular weights were obtained from a plot of $\log_{10}$ MW versus mobility using cytochrome *c* (13 500), chymotrypsinogen (25 000), aldolase (40 000), ovalbumin (45 000), catalase (60 000), bovine serum albumin (67 000) and rabbit myosin heavy chain (200 000). The mobilities of all chains were relative to aldolase. Myosin preparations from the following mice: a) 9 week normal adult 129 RE; b) foetal Banded-Dutch; c) 9 week dystrophic adult 129 RE; d) 9 week normal adult Banded-Dutch with marker aldolase. HC – heavy chain; L_1, L_2 and L_3 – light chains; B, H, and J approximate to bands described by Starr and Offer [6]; AL – aldolase.

Table 1

Specific Ca^{2+}-ATPase activities of myosins isolated from skeletal muscle of adult, foetal and dystrophic mice.

	Specific Ca^{2+}-ATPase activities (μmol ADP/mgm myosin/min)				
	Adult	Dystrophic		Adult	Foetal
1	0.35	0.26	8	1.00	0.57
2	0.39	0.26	9	0.72	0.18
3	0.79	0.53	10	0.82	0.40
4	0.37	0.35			
5	0.23	0.27			
6	0.90	0.66			
7	1.00	0.44			
X	0.58	X 0.40	X	0.85	X 0.38

Paired comparison

t-test: $t_6 = 2.386, p \sim 0.06$ $t_2 = 12.044, p < 0.01$

Each row represents the values obtained using myosin extracted on the same day from normal adult mice and dystrophic littermates (1–7) or from normal adult mice and foetuses (8–10), except the values in row 10 which do not formally constitute a pair, but were treated as such for the purposes of the paired comparison *t*-test.

Ca^{2+}-activated ATPase activity of myosin was determined in 0.5 M KCl, 0.6 mM ATP, 0.6 mM $CaCl_2$, 0.25 mM dithiothreitol and 25 mM Tris–HCl pH 7.2 at 28°C. The ADP released was determined by measuring the reduction in absorbance at 366 nm after incubation with NADH in the presence of phosphoenolpyruvate, pyruvate kinase and lactic dehydrogenase at pH 7.2 [9]. (Boehringer ADP–AMP Test combinations enabled direct estimation of ADP up to 0.7 mM under the conditions described). For determination of Mg^{2+}-activated ATPase, $CaCl_2$ was replaced by 0.6 mM $MgCl_2$. Determination of ADP by the coupled reactions was not affected by the small amounts of Ca^{2+} and Mg^{2+} carried over from the incubation mixture. Specific ATPase was measured with myosin at concentrations of 0.05–0.15 mg/ml of incubation mixture and ADP production was measured for linearity in each experiment. Myosin concentrations were determined from adsorbancy measurements with corrections for Rayleigh scattering, assuming that myosin $E^{1\%}_{1\,cm} = 5.60$ at 279 nm [10].

chromatography on DEAE-Sephadex A-50 to obtain the myosin which was released at a KCl concentration of 0.15–0.20 M. All solutions used after stage (2) contained 0.5 mM dithiothreitol.

Ca^{2+}-ATPase activity was found to be associated exclusively with the 0.15–0.20 M KCl chromatography fraction and SDS-gel electrophoresis showed band patterns (fig. 1) similar to those published for rabbit myosin [6]. The myosin had an $A_{280/260}$ ratio of 1.60–1.70 and no difference was found between

Table 2
Relationship between stoichiometry of light chains and specific Ca^{2+}-ATPase of adult, foetal and dystrophic myosin.

	Ca^{2+}-ATPase μmoles/mgm/min	% of total protein			
		Light chains	L_1	L_2	L_3
Adult	0.644 ± 0.149	23.2 ± 1.5	9.6 ± 0.9 (1.8)	9.7 ± 0.8 (2.1)	3.9 ± 0.6 (1.0)
Dystrophic	0.430 ± 0.084	18.8 ± 1.1	8.1 ± 0.6 (1.5)	8.1 ± 0.8 (1.8)	2.5 ± 0.2 (0.6)
Foetal	0.377 ± 0.113	22.9 ± 2.0	12.1 ± 0.8 (2.3)	8.3 ± 1.2 (1.8)	2.5 ± 0.1 (0.6)

The value for the % of each light chain in myosin was obtained from densitometry of gels using a Joyce–Loebl densitometer. The recorded peaks from the chart paper were traced, cut out and weighed. It was assumed that the area under each peak was proportional to its protein content and that each component bound dye to the same extent. The moles of light chain (values in parentheses) were determined assuming a value of 470 000 daltons for all myosins and molecular weights of 25 000, 21 600 and 18 400 for L_1, L_2 and L_3. The values represent the means ± standard error of the mean using five adult, four dystrophic and three foetal myosin preparations.

the ratios of adult, foetal and dystrophic myosin preparations. Specific Mg^{2+}-ATPase activity was low in all myosin preparations indicating the absence of actomyosin contamination.

3. Results

The specific Ca^{2+}-ATPase levels of dystrophic and foetal myosin were on average 69% and 44% respectively of the adult level determined in the same experiment (table 1). The ATPase levels of normal adult myosin, dystrophic myosin and foetal myosin preparations varied between 1.0 and 0.23, 0.66 and 0.26, and 0.57 and 0.18 μmol ADP/mg myosin/min respectively. Nevertheless, despite this variation, in the case of dystrophic myosin the difference is only just short of formal significance at the 5% level, tested as paired comparisons (t_6 = 2.386) suggesting very strongly that dystrophic myosin had a lower specific Ca^{2+}-ATPase activity. In the case of foetal myosin, the difference was significant at the 1% level using the same test (t_2 = 12.044).

Foetal and dystrophic myosin preparations contained three light chains (fig. 1) with molecular weights indistinguishable from adult myosin light chains L_1 (25 000), L_2 (21 600) and L_3 (18 400). This was established by electrophoresing marker aldolase on the same gel as the myosin samples, and by co-electrophoresing mixtures of the three types of myosin. Foetal and dystrophic myosin differed from adult myosin in the relative amount of the light chains (table 2). The precentage of total light chains in foetal myosin did not differ from the adult (23% in both cases). However, the relative amount of L_1 increased and L_2 and L_3 chains decreased relative to adult myosin. The total light chains made up only 18–19% of dystrophic myosin and the relative amounts of L_1, L_2 and particularly L_3 were decreased. The differences between the L_3 content of adult, dystrophic and foetal myosin were also tested as paired comparisons where paired observations were available. The difference between normal adult and dystrophic myosin L_3 content, while it falls short of formal significance (D = 1.38 ± 0.70 (4)), clearly suggests that there is a decreased amount of L_3 in dystrophic myosin, particularly as it is supported by two comparisons involving normal adult and foetal myosins (D = 2.6 ± 0.85 (2)).

It is unlikely that the reduction in both the total light chain content and the specific Ca^{2+}-ATPase activity of dystrophic myosin was due to proteolytic activity during the extraction procedure because of the following evidence. After the initial extraction of dystrophic muscle, the extraction medium was dialyzed against 14 vol of H_2O to lower the ionic strength and precipitate the myosin which was removed by centrifugation. The extraction medium was then brought back to the original ionic strength (original volume) by dialysis and reused to extract normal muscle myosin. The heavy and light chain composition and specific Ca^{2+}-ATPase activity of this myosin

was the same as for normal myosin prepared in the usual way.

4. Discussion

The results (table 2) indicate that adult mouse myosin (from the mixed skeletal muscle of the lumbar region and hind limbs) has five light chains $(L_1)_2$ $(L_2)_2$ L_3 per molecule. Previous evidence has indicated that myosin from fast muscles of rabbit and chicken contains a total of four light chains L_1 $(L_2)_2$ L_3 per molecule [11, 12]. The L_1 and L_3 chains of rabbit myosin do not always show equimolar stoichiometry and it has been suggested that there are two different populations of myosin molecules with either two L_1 or two L_3 chains [13, 14]. These populations of myosin molecules may be present in different proportions which would explain the apparent non-equimolar stoichiometry. It seems unlikely that the light chain composition of adult mouse myosin can be explained by assuming that there are two populations of myosin molecules with either L_1 or L_3 chains, because it was observed that for four different myosin preparations, while the estimate for the number of L_1 chains varied between 2.2 and 1.3, the value for L_3 remained fairly constant between 0.85–1.1 (means only are shown in table 2).

The fact that each mole of foetal myosin contained 0.6 mole of the light chain L_3 (table 2) suggests that foetal myosin preparations may contain some myosin molecules without any L_3 chains at all. If it is assumed that foetal myosin has a chain composition of $(L_1)_3$ $(L_2)_2$ and adult myosin $(L_1)_2$ $(L_2)_2$ L_3, and that the proportion of each myosin changes as development progresses, and appropriate mixture would give the observed 19-day foetal myosin composition $(L_1)_{2.2}$ $(L_2)_2$ $(L_3)_{0.6}$. An alternative explanation is that during the course of development either the L_3 chain is added to a prototype myosin molecule containing $(L_1)_2$ $(L_2)_2$ only or the adult myosin molecule containing $(L_1)_2$ $(L_2)_2$ L_3 is gradually substituted for the primitive form. Whichever process takes place, the end result is myosin with a higher specific Ca^{2+}-ATPase activity.

Dystrophic myosin is similar to foetal myosin in having c.a 0.6 moles of light chains L_3 per mole and a low level of specific Ca^{2+}-ATPase and in this way resembles the myosin of dystrophic rabbits [2]. However unlike dystrophic rabbit myosin, dystrophic mouse myosin has a decreased total light chain content which may also affect the level of Ca^{2+}-ATPase activity. A previous report from this laboratory [15] indicated that in some myosin preparations from dystrophic mice, the L_2 and L_3 chains were undetectable, there was degradation of the heavy chain, and the specific Ca^{2+}-ATPase activity was at a low level. When myosin was prepared from normal muscle using extraction medium previously employed to treat dystrophic muscle, it showed similar degradation of the heavy chain and absence of the L_2 chain, indicating that normal myosin had been partly degraded by proteolytic enzymes derived from the dystrophic muscle [15]. However, the L_3 chain and the specific Ca^{2+}-ATPase activity were not affected by this treatment [15] suggesting that the presence of the L_3 chain was crucial for high specific Ca^{2+}-ATPase activity. In the present investigation, there was no evidence for proteolytic degradation of dystrophic myosin, which may be due to the quite different techniques used for myosin purification.

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